Supporting Information

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SI Materials and Methods

Viruses. Lymphocytic choriomeningitis virus (LCMV) Armstrong (ARM) and clone 13 (Cl13) stocks were prepared by serial passage in BHK-21 cells, and titers were quantified as described (1). Recombinant variants were generated by using reverse genetic technology (2, 3). Virus titers in serum of infected mice were determined by plaque assays on VeroE6 cells (5).

Mice. NZB, C57BL/6, and BALB/c mice were purchased from the Jackson Laboratory. Generation of congenic NZB mice (Ifnar1<sup>-/-</sup>, Ifnb<sup>-/-</sup>, Tlr3<sup>-/-</sup> and Unc93b1<sup>−/−</sup>) was as described (6–8). Mice were housed under specific pathogen-free conditions, and all experimental protocols were performed according to the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals (9) and approved by The Scripps Research Institute Animal Care and Use Committee. For all experiments, 8- to 10-wk-old mice were used. Although both male and female NZB mice were found to be susceptible to Cl13-induced death, all experiments were performed with male mice. Estimation of group sizes was based on previous experience with LCMV-infected mice, without a priori determination by power calculation. Individual mice were randomly assigned to experimental groups and analyzed under identical experimental conditions but without blinding. Infection with Cl13, ARM, or recombinant LCMV variants was performed by i.v. injection of 5 x 10<sup>6</sup> plaque-forming units (PFU) per mouse. When indicated, mice were treated intraperitoneally (i.p.) with a blocking monoclonal anti-IFN-α receptor (IFNAR) antibody (clone MAR1-5A3; Leinco Technologies), as described (7, 10), by using 500 μg at the first injection (in most instances given 1 d before infection unless otherwise stated) followed by 250 μg three times per week for 2 wk. For CD4<sup>+</sup> T-cell depletion, mice were treated i.p. with the monoclonal GK1.5 antibody (clone Gk1.5; BioXCell) and on days 1 and 0 (500 μg) and on days 3 and 5 postinfection (pi) (250 μg). Similar doses and schedules were used for depletion of CD8<sup>+</sup> T cells using the monoclonal YTS69 antibody. Efficiency of CD4<sup>+</sup> and CD8<sup>+</sup> T-cell depletion was >98%, as assessed by FACS analysis of blood and spleen cells on day 4 or in vitro cytotoxic T lymphocyte (CTL) assay on day 6 pi (Fig. S3). For blood and serum analysis, blood was drawn by retroorbital puncture. Platelet counts were determined by using an automated cell counter (Hemavet 950FS; Drew Scientific). Serum was isolated by low-speed centrifugation and frozen until use.

Mouse Chimera. Bone marrow cells were extracted from femurs of NZB mice (8 wk old) and filtered through a 100-μm mesh screen, and red blood cells were lysed as described (11). Isolated bone marrow cells (1–3 x 10<sup>5</sup>) were adoptively transferred into lethally irradiated (1,100 rad) WT or Ifnar1<sup>-/-</sup> NZB mice. The transferred mice (WT→WT and WT→Ifnar1<sup>-/-</sup> bone marrow chimeras) were maintained on water supplemented with antibiotics for 2 mo to prevent opportunistic infections. Reconstitution was verified 2 mo after bone marrow transfer by FACs analysis of blood cell, by using anti-IFNAR antibody to differentiate WT from Ifnar1<sup>-/-</sup> cells. Efficiently reconstituted mice were infected with Cl13 (2 x 10<sup>6</sup> PFU) and followed for survival.

Tissue Histology and Cell Isolation. Spleen, lungs, heart, brain, liver, and kidneys were harvested from infected and control mice, placed in PBS-buffered formalin, and blocked in paraffin, and 10-μm tissue sections were histochemically stained (hematoxylin and eosin or Masson’s Trichrome). Single-cell suspensions were prepared from spleen or blood as described (12). Lungs harvested from PBS-perfused mice were mechanically diced into small pieces, suspended in RPMI containing collagenase D (Sigma; 2 mg/mL), DNase I (bovine pancreas grade II; Roche; 0.1 mg/mL), and trypsin inhibitor (type II-s; Sigma; 1 mg/mL), and incubated for 1 h at 37 °C. After additional mechanical disruption through a 100-μm filter, red blood cells were lysed by exposure to lysis buffer (0.02 M Tris-HCl, 0.14 M NaCl, pH 7.5). Lung-infiltrated inflammatory cells were further purified from this cell suspension by centrifugation in 35% (vol/vol) PBS-buffered Percoll (GE Healthcare Life Sciences).

Vascular Leakage. Infected and control mice (day 5.5 pi) were i.v. injected with 200 μL of Evan’s blue dye (0.5% in PBS). After 20 min, mice were lethally anesthetized and perfused by intracardiac injection of 10 mL of PBS, and lungs were harvested. Extravascular Evan’s blue was then extracted by overnight incubation in formamide at 56 °C and quantitated by photometrical analysis (620 nm).

Bronchoalveolar Lavage Fluid Analysis. The trachea of euthanized mice was exposed, transected, and intubated with an 18-gauge needle. One milliliter of PBS supplemented with Complete Mini, EDTA-free Protease Inhibitor Mixture (Roche) was infused and recovered four times. The recovered bronchoalveolar lavage fluid (BALF) was centrifuged (3,000 x g, 3 min) and frozen until use. Total protein levels were determined by using mouse IFN-α ELISA kit (Thermo Scientific). IgM levels were quantified by using the mouse IgM quantitation kit (Bethyl Laboratories), and lactate dehydrogenase enzymatic activity was determined by using the Cytotox 96 nonradioactive cytotoxicity assay (Promega).

ELISA. Cytokine and chemokine levels in serum and BALF were assessed by using CCL2 (MCP-1), CCL5 (RANTES), CXCL10 (IP-10), IL-6, TNF-α, and IL-10 Duoset ELISA kits (R&D Systems); the CCL3 and CXCL2 Quantikine kits (R&D Systems); the VeriKine Mouse IFN-Alpha Kit (R & D Systems); and the IFN-α ELISA kit (PBL InterferonSource).

CTL Analysis. Spleen cells from infected and control mice were evaluated for CTL activity in a standard 51<sup>Cr</sup> release assay as described (1). Briefly, MC57 (H-2<sup>b</sup>) and BALB/cI7 (H-2<sup>b</sup>) target cells were infected (or not) with either ARM or Cl13 for 24 h at a multiplicity of infection of 1. These cells were subsequently labeled with 51<sup>Cr</sup> for 1 h at 37 °C, washed, and incubated with spleen cells (ratio effector:target = 50:1) in triplicate for 5 h. Cell lysis was quantified by assaying supernatants for 51<sup>Cr</sup> using a γ-counter, and values were expressed as percent lysis, using Nonidet P-40-treated cells as 100% lysis controls.

FACS. For surface staining, cells were incubated with fluorochrome-labeled antibodies specific for mouse CD4 (L3T4), CD8a (53-6.7), CD44, CD62, CD137 (4-1BB), PD-1 (RMPi-30), TIM-3 (RMT3-23), LAG-3 (C9B7W), CD45, CD31, Podoplanin (gp38, PD-L1, PD-L2, MHC-I, MHC-II, CD69, CD11b (M1/70), F4/80, Ly6G, CD3, and NK1.1, as described (10–13). Cells were then washed with PBS and fixed with 4% paraformaldehyde. For tetramer staining, mononuclear cells were incubated with MHC-I tetramers (H-2L<sup>d</sup>/NP<sub>118-126</sub>), before any additional surface staining. Biotinylated MHC-I monomers were
obtained from the NIH Tetramer Core Facility and tetramerized by using streptavidin–APC (Invitrogen). For intracellular staining, cells were fixed, permeabilized with 2% saponin or permeabilization solution (BD, Pharmingen), and stained with antibodies to LCMV NP (VL-4) or granzyme B (MHGB04; Invitrogen). For intracellular antibody detection, spleen cells were first restimulated in vitro for 5 h with 2 mg/mL H-2Ld-restricted LCMV peptide NP118–126 in the presence of 4 mg/mL brefeldin A (Sigma). Cells were then fixed, permeabilized with 2% saponin, and stained intracellularly with antibodies to IFN-γ (XMG1.2) and TNF-α (MP6 XT22). Surface and intracellularly stained cells were acquired on a BD FACSDiva-driven BD LSR II or a four-color FACSCalibur flow cytometer. Data were analyzed by using FlowJo software (Treestar).

**Statistical Analysis.** Group comparisons were analyzed by unpaired two-tailed Student t test. Survival was analyzed by Kaplan–Meier plot and log-rank test. *P* < 0.05 was considered significant.


**Fig. S1.** Virus titers in serum of Cl13- or ARM-infected NZB and BALB/c mice. NZB and BALB/c mice were infected with either Cl13 or ARM (2 × 10⁶ PFU per mouse). Virus titers in serum were determined at days 4 and 6 pi (*n* = 3 or 4 mice per group; Student t test). *P* < 0.05; **P** < 0.005.

**Fig. S2.** Increased lung pathology caused by Cl13 in NZB mice compared with C57BL/6 and BALB/c controls. NZB, C57BL/6, and BALB/c mice were infected with Cl13 (day 0 pi) and killed (day 6 pi), and lung tissue sections were histochemically stained with Masson’s trichrome. Data are from two experiments (*n* = 3 or 4 mice per group). [Scale bars, 100 μm (Upper) or 20 μm (Lower).]
Fig. S3. Efficiency of CD4<sup>+</sup> and CD8<sup>+</sup> T-cell depletion in Cl13-infected NZB mice. NZB mice were treated (or not) with depleting anti-CD4 or anti-CD8 antibodies (days −1, 0, 3, and 5 pi) and infected with Cl13 (day 0). (A) At day 4 pi, blood cells from treated and control mice were examined by FACS for the presence of CD4<sup>+</sup> and CD8<sup>+</sup> T cells. (B) At day 6 pi, mice were killed, and spleen cells were assessed for CTL activity against Cl13-infected BALB/C17 cells as targets. Data are representative of two experiments (n = 3 or 4 mice per group; Student t test). **P < 0.005.

Fig. S4. IFN-α production in Cl13- and ARM-infected NZB mice. NZB mice were infected with Cl13 or ARM, and serum levels of IFN-α were determined by ELISA at days 0, 1, 2, 3, and 6 pi. Data are representative of two experiments (average ± SEM; n = 3 or 4 mice per group).

Fig. S5. Efficiency of antibody-mediated IFNAR blockade in Cl13-infected NZB mice. NZB mice were infected with Cl13 (day 0) and treated with the following: nine injections of anti-IFNAR antibody between days 0 and 15 pi (A); six injections of anti-IFNAR antibody starting at 24, 60, or 72 h pi until day 13 pi (B); and a single injection of anti-IFNAR antibody on day 1 pi (C). Gray area in graphs defines the time window of anti-IFNAR treatment. Data are from one to three experiments (n = 3 or 4 mice per group).

Fig. S6. IFN-I–independent up-regulation of the inhibitory molecules PD-L1 and -L2 in lung endothelial cells of Cl13-infected NZB mice. NZB mice were infected with Cl13 (day 0) and treated or not with blocking anti-IFNAR (days −1, 2, and 4 pi). Expression of PD-L1 and -L2 in lung vascular endothelial cells (CD45<sup>−</sup>CD31<sup>+</sup>Podo/Epithelial gp38<sup>−</sup>) was determined by FACS on day 5.5 pi. Numbers indicate frequencies (average ± SEM) of PD-L1<sup>+</sup>PD-L2<sup>−</sup> and PD-L1<sup>+</sup>PD-L2<sup>+</sup> cells (n = 3 or 4 mice per group).
Fig. S7. Lung endothelial cell infection and MHC-I up-regulation in lung of CI13-infected BALB/c mice. (A) Intracellular LCMV in lung vascular endothelial cells (CD45–CD31+Podoplanin/gp38–) from control and infected BALB/c mice at day 6 pi was assessed by FACS. Numbers indicate frequencies (average ± SEM) of infected cells (n = 3 mice per group). (B) Lung endothelial cells (CD45–CD31+Podoplanin/gp38–) of CI13-infected BALB/c mice were enumerated at day 5.5 pi, and MHC-I expression was quantified (MFI) by FACS (average ± SEM; n = 3 mice per group; Student t test). **P < 0.005.

Table S1. Inoculation of 2 × 10^6 PFU of LCMV CI13 generates virus-specific CTLs in NZB mice

<table>
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<tr>
<th>Mouse</th>
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Data are representative of two independent experiments (n = 4 mice per group).
* Mice were treated (or not) with anti-IFNAR blocking antibody (day 0).
† Spleen cells were analyzed on day 6 pi for cytotoxic activity against LCMV-infected targets; H-2d targets were BALB/c7 cells, and H-2b targets were C57BL/6 cells.
‡ Percentage of total release (average ± SEM).